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DUST-COUNTING CELLS

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BY

CARLTON E. BROWN, R. L. BEATTY, AND T. B. KIRBY

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DUST-COUNTING CELLS[1]

By Carlton E. Brown,[2] R. L. Beatty,[3] and T. B. Kirby[4]

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1/ The Bureau of Mines will welcome reprinting of this paper provided the following footnote acknowledgment is used: "Reprinted from Bureau of Mines Information Circular 7331."

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INTRODUCTION

This report contains information on the cells or containers used to hold a known small depth (usually 1 mm. or less) of the dust-containing liquid from impinger,[5][6] filter-paper,[7] and other samples collected from the air while the particles revealed by a microscope (equipped with a 16-mm., 0.25- or 0.30-N. A. objective and ordinarily used with axial light-field illumination) are counted in order to obtain an index of the concentration of dust in the sampled air. Information is given on such subjects as the kinds of cells available, kinds used, actual depths of cells, and construction of simple, inexpensive cells.

ACKNOWLEDGMENTS

The interest, encouragement, and assistance received from H. H. Schrenk, chief chemist of the Health Division, in the preparation of this report are gratefully acknowledged, as is all other assistance received.

COUNTING DUST IN A LIQUID MEDIUM

Determination of the amount of unhygienic dust in samples collected from the air by counting the particles (revealed by a particular microscopic system) in a liquid medium was suggested as early as 1908.[8] The procedure suggested was patterned after one that had been used for at least 19 years (since 1889) in counting various organisms in water.[9] The procedure was adopted by the American Public Health Association[10] [11] and others in the United States for determinations on samples collected from the air by

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5/ Brown, C. E., and Schrenk, H. H., A Technique for Use of the Impinger Method: Bureau of Mines Inf. Circ. 7026, 1938, 20 pp.

6/ Greenburg, L., and Bloomfield, J. J., The Impinger Dust Sampling Apparatus as Used by the United States Public Health Service: Pub. Health Repts., vol. 47, 1932, pp. 654-675.

7/ Brown, C. E., Filter-Paper Method for Obtaining Dust-Concentration Results Comparable to Impinger Results: Bureau of Mines Rept. of Investigations 3788, 1944, 20 pp.

8/ Winslow, C.-E. A., A Method for Determining the Number of Dust Particles in Air: Am. Jour. Pub. Hyg., vol. 19, N. S., vol. 5, 1909, pp. 87-88, and Eng. News, vol. 60, Dec. 31, 1908, p. 748. (Abstract of a paper read before the Laboratory Section of the Am. Pub. Health Assoc. at Winnipeg, Canada, August 1908)

9/ Whipple, G. C., The Microscopy of Drinking Water: John Wiley & Sons, Inc., 1914, 3d ed., 409 pp. (or any other edition).

10/ American Public Health Association, Report of the Committee on Standard Methods for the Examination of Air: Am. Jour. Pub. Hyg., vol. 20, N. S., vol. 6, 1910, pp. 346-360.

11/ American Public Health Association, Final Report of the Committee on Standard Methods for the Examination of Air: Am. Jour. Pub. Health, vol. 7, 1917, pp. 54-72.

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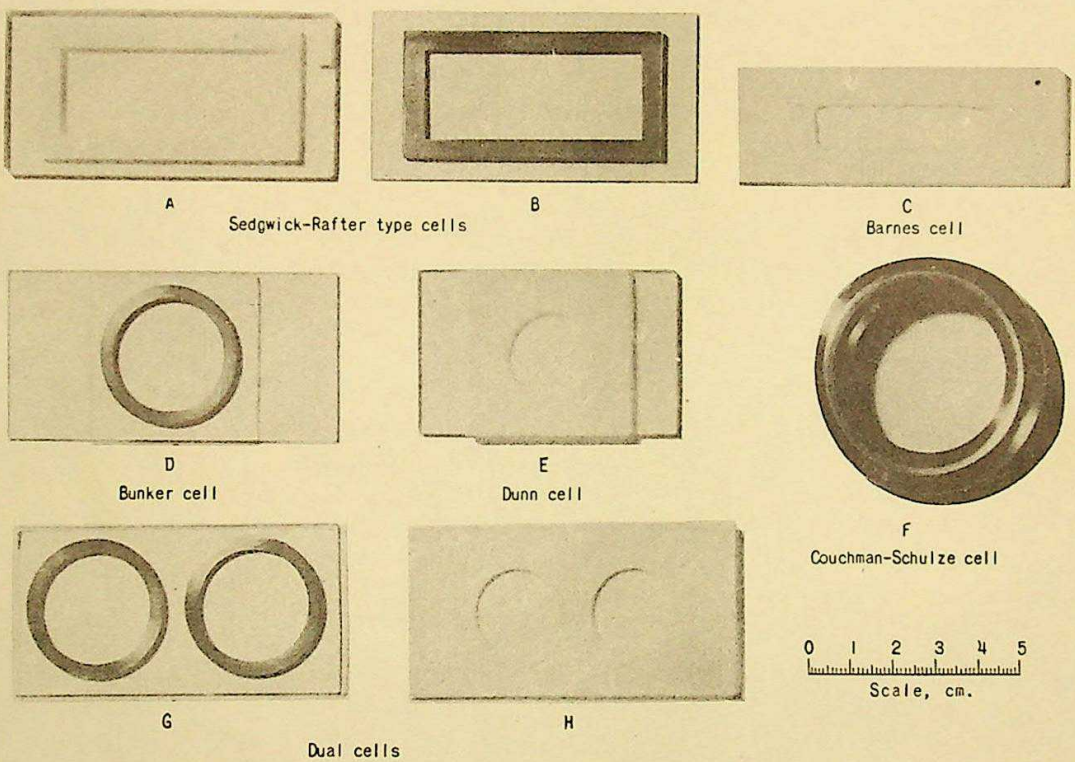

Figure 1.—Counting cells one millimeter in depth.

such samplers as the Palmer apparatus,[12] sugar-tube,[13] impinger,[13] and filter-paper.[14] This counting procedure, without essential modification, is still employed in this country.

Counting procedures patterned after those for counting cells in blood (or at least using the same type of counting cells) were used in South Africa[15] as early as 1915. These procedures apparently have been used to a limited extent in this and other countries. The procedure reportedly was discontinued in South Africa after a short time because of the small amount of dust in the samples.[16]

The counting procedures consist essentially of the following operations: (1) Obtaining the material or objects (dust or dust particles) to be counted in a measured or known volume of liquid, (2) distributing the dust uniformly throughout the liquid by shaking or other means, (3) transferring a portion or portions of the liquid to suitable cells or containers, (4) allowing the cells to stand to permit the dust to settle to the floor of the cell (where the particles can be seen more easily than while suspended in the liquid), and (5) counting the particles (revealed by the microscopic system used) in measured portions of the liquid.

CELLS

All dust-counting cells have parallel plane glass tops and bottoms to permit microscopic observation of the dust particles contained in them. Most cells are on or in glass slides of convenient size (usually 25 x 75 mm. or 1 x 3 inches) for use with a microscope. Some cells are completely closed when in use and others are open on two sides, the liquid being held in them by surface tension. Open-sided cells usually have relatively deep trenches at the edges of the cell floor to facilitate filling of the cell and to reduce the effect of evaporation upon the small portion of the liquid above the cell floor.

The original cells used in the United States and most that have been used since have been 1 mm. in depth (the important dimension). Some cells 0.1 mm. and others 0.25 mm. deep also have been used to a limited extent. Cells 0.2, 0.4, 0.7, 0.9, 2, and 3 mm. deep have been used mainly in experimentation. The actual depth of cells may be quite different from the

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12/ Palmer, G. T., A New Sampling Apparatus for the Determination of Aerial Dust: Am. Jour. Pub. Health, vol. 6, 1916, pp. 54-55.

13/ Katz, S. H., Smith, G. W., Myers, W. M., Trostel, L. J., Ingels, M., and Greenburg, L., Comparative Tests of Instruments for Determining Atmospheric Dusts: Pub. Health Bull. 144, 1925, 69 pp.

14/ Brown, C. E., Work cited in footnote 7.

15/ Moir, J., Recent Investigations on Dust in Mine Air and the Causation of Miners' Phthisis: Jour. Chem., Met. and Min. Soc. S. Afr., vol 16, 1915, July-Aug., pp. 1-8.

16/ Miners' Phthisis Prevention Committee, Final Report: Johannesburg, 1919, 110 pp.

stated depth, hence the depth should be measured (by a graduated fine focus on a microscope, a micrometer caliper, or other means) before the cells are used. Cells deeper than the stated depth may be reduced by rubbing the tops of their walls on a layer of moistened fine abrasive, such as No. 600 carborundum on glass. Opticians and other workers in glass probably would do the work of reducing cells with glass walls for a reasonable fee.

Cells 1 mm. in Depth

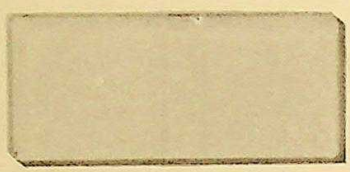
Figure 1 shows various kinds of cells 1 mm. in depth. They differ in such things as number and shape of containers, number of parts, and kind of walls. Most have one cell or container; others have two. Some consist of two parts (cell and cover), others of three (cell walls, top, and bottom), and one of four (glass top and bottom, metal cell walls, and metal clamping ring). The cells or containers of some are rectangular and of others circular. The walls of some are metal (usually brass, but occasionally stainless steel) and others are glass. The walls of most three-piece cells are glass. Richardson, Warren and Williamson[17] recently described a three-piece cell with metal walls. Metal walls of two-piece cells usually are cemented onto the glass base, and glass walls of such cells are either cemented or fused to the glass base.

"A" in figure 1 shows the first type of cell used in the United States for counting dust. It was developed about 1889 by Prof. W. T. Sedgwick of Massachusetts Institute of Technology and Geo. W. Rafter of Rochester[18] for counting plankton (microscopic organisms) in water. Prof. Sedgwick suggested the use of a cell bounded by a brass rim and having an area of 1,000 sq. mm. ruled by a dividing engine into 1,000 squares. Rafter suggested the use of an ocular micrometer instead of the ruling on the floor of the cell. Whipple[18] stated about this cell:

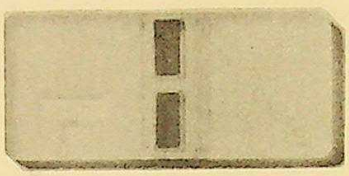
> The cell (Sedgwick-Rafter) *** is made by cementing a brass rim to an ordinary glass (microscope) slip. The cell originally was rectangular. Its internal dimensions were length 50 mm., width 20 mm., and depth 1 mm. It, therefore, has an area of 1000 sq. mm. and a capacity of 1 cc. A thick cover-glass (No. 3) having dimensions equal to those of the outside of the brass rim (55 mm. by 25 mm.) forms a roof to the cell.
>
> It is not necessary to use a rectangular cell. A circular cell is equally satisfactory, is much cheaper, and is easier cleaned. The capacity of the cell is immaterial, but a volume of about one cubic centimeter is most convenient. It is necessary, however, that the depth be exactly one millimeter.

17/ Richardson, A. S., Warren, J. W., and Williamson, W. C., Some Sources of Error in Dust Sampling Work Done By the Impinger Method: Trans., 33d Nat. Safety Cong., 1945, pp. 354-358.

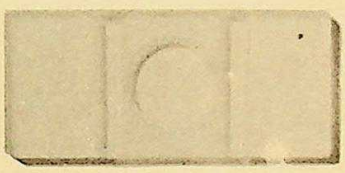
18/ Whipple, G. C., work cited in footnote 9.



Levy counting chamber with improved Neubauer double ruling. (For counts on blood cells.)

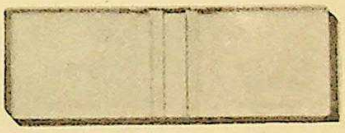


Spencer Bright-line counting chamber with improved Neubauer double ruling. (For counts on blood cells.)



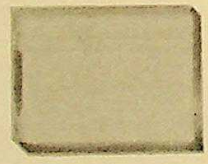
Howard counting chamber. (For counts on molds, yeasts, etc.)

0.1-mm. deep cells

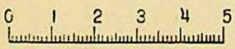


Levy counting chambers with Fuchs-Rosenthal ruling. (For counts on cells in body fluids other than blood.)

0.2-mm. deep cells



Hatch cell. (For counts on dust.)
0.25-mm. deep cell



Culture slide. (For counts living specimens in very large hanging drops or in agar blocks.)
3-mm. deep cell

Figure 2.—Counting cells other than one millimeter in depth.

The Bureau of Mines and probably many other organizations and individuals have made Sedgwick-Rafter-type cells by cementing (rectangular and circular) metal walls to ordinary or thick (about 2 mm.) microscope slides of suitable size. Microscope slides found by dark-field microscopic examination to be relatively free from pits, scratches, and other imperfections should be used. Nineteen Brown & Sharpe-gage sheet metal has been used in making some of these walls. The thickness (0.91 mm., or 0.03589 inch) of this sheet allows 0.09 mm. or 0.0035 inch for the thickness of the cement. Cements insoluble in water and alcohol (ethyl, iso-propyl, and propyl) have been used, as these liquids are used for dust samples. Many of the readily available adhesives, such as glue, rubber cements, plastic solutions (collodion, polystyrene, Duco household cement, So-Lo cement, vinyl chloride in dioxane, and Hercules stabelite in chloroform), and sodium silicate (water glass), alone or in combination with various fillers, have been tried for holding the metal walls to the glass slides, but few have been found to be satisfactory under the conditions of use. Rubber cements (such as B. F. Goodrich's Vulcalock) seem to be satisfactory. Some of the other adhesives might be satisfactory under conditions other than those of the preceding trials. Help in selecting and using suitable adhesives may be obtained from various manufacturers,[19] some of whom state that they market or will make an adhesive for every purpose.

The depth of home-made cells as well as purchased cells should be measured carefully before use at several places (one or more places on each side of rectangular cells). The depths of 24 of one of the types of cells shown in figure 1, purchased by the Bureau of Mines, were found to range from 1.15 to 1.50 mm. The depths of some of the cells were found to vary as much as 0.11 mm. in different parts of the cell. The dealer was notified. He replaced the cells, but stated that his manufacturing processes did not permit tolerances closer than 1 $\pm$ 0.20 mm. Richardson, Warren, and Williamson[20] found that the depth of 11 cells obtained from a supply house varied from 0.945 to 1.195 mm. Counts theoretically vary inversely with the depth of the cell. Thus, use of the stated instead of the actual depth of the cell as the basis for calculations may lead to significant errors in results. If a stated depth of 1.0 mm. instead of the actual depth of a 1.20 mm. cell is used, results will be 20 percent too high.

"B" in figure 1 shows a foreign-made Sedgwick-Rafter-type cell with glass walls. The walls, consisting of four glass strips, are fused to the thick glass base. Water, alcohol, and other liquids used in dust samples do not seem to affect this fused connection. Leakage at the corners or junction between the glass strips is one objection to this type of cell.

"C" in figure 1 shows a cell developed about 1935 by E. C. Barnes[21] industrial hygienist of the Westinghouse Electric & Manufacturing Co. The cell consists of three plain microscope slides (obtainable from optical

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19/ See any of the various purchasing guides for lists of manufacturers.
20/ Richardson, A. S., Warren, J. W., and Williamson, W. C., Work cited in footnote 17.
21/ Personal communication.

companies and laboratory-supply houses for about 1 cent each), one of which has a centrally located rectangular opening cut through it by abrasive blasting. Circular openings probably could be blasted, cut, or drilled about as easily. These slides are approximately 1 mm. thick. The slide with the rectangular opening forms the walls, one of the other slides the bottom, and the third slide the cover. The walls are not (but could be) cemented to the bottom slide. Surface tension of the liquid holds it in the cell. The all-glass construction of this cell and the absence of any cement permit the use of any liquid (except fluorides) in the cell or in cleaning the cell. Cleaning of this cell is simpler than cleaning one that can not be disassembled.

"D" in figure 1 shows a circular type of cell developed many years ago by Dr. J. W. M. Bunker.[22] The cell shown has a brass ring about 0.91 mm. thick cemented (with B. F. Goodrich's Vulcalock) onto a microscope slide. These rims are easier and cheaper to make than the rectangular ones. The predominance of the more expensive and harder-to-clean rectangular cells over the circular ones is somewhat surprising. Scarcity of information on circular cells in industrial-hygiene literature and the usual dust-counting directions for counting (in each of two cells from the same sample) five fields - one near the middle and one near each corner - probably have been partly responsible for the relatively little use of circular cells in dust counting. Richardson, Warren, and Williamson's[23] cell is similar, but the rim is not cemented to the slide.

"E" in figure 1 shows a three-piece, all-glass, circular cell described by Dunn.[24] This cell is similar in principle to the Barnes cell.

"F" in figure 1 shows a circular cell consisting of a circular glass disk (goggle lens) clamped in a two-piece aluminum holder, which provides the 1-mm.-deep cell walls. The glass disk may be removed from the holder for cleaning by unscrewing the two parts, and the various parts may be cleaned by immersion in a suitable liquid or by other means. This cell was described by Couchman and Schulze[25] in 1938.

"G" and "H" in figure 1 show circular cells having two cells or containers.[25a] "G" is a two-piece cell having two circular brass rims cemented (with Vulcalock) on to a microscope slide. "H" is a three-piece cell having two circular holes in a microscope slide. These cells (suggested by C. E. Brown, one of the authors) have some of the features of certain of the

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22/ Whipple, G. C., Work cited in footnote 9.

23/ Richardson, A. S., Warren, J. W., and Williamson, W. C., Work cited in footnote 17.

24/ Dunn, K. L., Note on an Improved Cell for Dust Counting: Jour. Ind. Hyg. Toxicol., vol. 21, 1939, pp. 202-203.

25/ Couchman, C. E., and Schulze, W. H., A Modified Cell for Dust Counting: Pub. Health Repts., vol. 53, 1938, pp. 348-350.

25a/ After this report was submitted for publication, the following article describing another dual counting cell came to our attention: McCormick, W. E., A Two-cavity Dust-counting Cells: Pub. Health Repts., vol. 60, May 4, 1945, pp. 489-491.

preceding cells and the additional advantage (to dust counting but not to blood counting or possibly other counting techniques) of essentially combining into one unit the two cells in which counts on impinger, filter-paper, and similar samples are made. The two containers can be filled at the same time (but they should not be filled from the same pipetteful of liquid), be placed under the microscope at the same time, be counted at the same time (preferably by alternately making counts on two or three fields in each cell until counts are made on the usual five fields in both cells), and be washed at the same time. Thus, the use of this dual cell eliminates all or most of the handling (filling, placing under the microscope, getting into focus, and cleaning) of a second cell. The depth of the two containers in the same slide, as in cell "H," is more likely to be the same than that of two other supposedly 1-mm.-deep cells. The cost of these cells should be less than that of two separate cells.

Some of the preceding cells (particularly "B" and "E") have been marketed by laboratory-supply houses, optical companies, and others. The price of cells like "A" and "B" has been about $7.

Cells Other Than 1 mm. in Depth

Cells other than 1 mm. in depth have been used or suggested for use in dust counting. Most of such cells have been less than a millimeter in depth. They have been used mainly to permit a reduction in the settling time (as a result of the shorter path through which the dust has to settle) and in dilution of concentrated samples (as a result of the smaller volume of liquid in the field examined).

The Bureau of Mines, in limited experimentation, has found indications that results on cells less than 1 mm. in depth do not agree well with those on cells 1 mm. in depth at equivalent settling times, but tend to vary inversely with the depth of the cell (or increase with a decrease in the depth of the cell). The exact explanation is not known, but difference in settling during the counting period may partly explain it. For example, if a cell 1 mm. in depth is allowed to settle 20 minutes before the count is started, and a cell 0.1 millimeter in depth is allowed to stand an equivalent time of 2 minutes, and the counts on both cells require 2 minutes, then the settling time for the first cell increased from 20 to 21 minutes (at the midpoint of the counting period), an increase of 5 percent, and that for the cell 0.1 mm. in depth increased from 2 to 3 minutes, an increase of 50 percent.

Cells deeper than 1 mm. have been used little, mainly because of the long time required for the microscopically visible dust to settle to the floor of the cell. However, cells 2 mm. in depth have been used by South African investigators to replace cells 0.1 mm. in depth that had been found unsatisfactory owing to the small amount of dust in the samples.26/

Figure 2 shows some of the cells other than 1 mm. deep used in counts on dust for regular and experimental purposes. All cells, except the last or

26/ Miners' Phthisis Prevention Committee, Work cited in footnote 16.

culture slide, are open on two sides, the liquid being held in these cells by surface tension. The floors of the open cells are higher than their immediate surroundings but lower, by the depth of the cell, than the supports for the cover glasses.

Cells 0.1 Mm. in Depth

The first two 0.1-mm.-deep cells shown were developed for use in counting cells in blood. Both cells are essentially dual, as each has two separate cell floors for use in obtaining check determinations and greater precision than is obtainable with single cells. These cells differ from the 1-mm.-deep dual cells ("G" and "H" in figure 1) in that both must be filled from the same portion of liquid, whereas the two cells in the 1-mm. dual cells should not be. Both of these 0.1-mm.-deep cells have rulings (improved Neubauer) on each of the two floors. The rulings in the first cells are in the glass, whereas those in the second (Spencer Bright-Line) are in a metal film applied by a sputter arc and fused into the glass. These rulings are not ordinarily used in counts on dust.

The third or last 0.1-mm.-deep cell (Howard counting chamber) was developed for counts on molds, yeasts, and other substances. The floor of the cell is circular and has no rulings.

Cells 0.1 mm. in depth have been used for regular counts on dust in the United States by Williams[27] and Ohlheiser and Lawrence,[28] in South Africa by Moir,[29] and probably in other countries. These cells have been used for experimental counts on dust in the United States by the Bureau of Mines, in Canada by Annetts and Leitch,[30] and probably by others in these and other countries.

Cells 0.2 Mm. in Depth

The two cells 0.2 mm. in depth shown in figure 2 were developed for counts on the cells in body fluids other than blood, as cells in cerebrospinal fluid. Both cells have a Fuchs-Rosenthal ruling on the floor.

These cells have been used to a limited extent for experimental counts on dust.

27/ Williams, C. R., A Method of Counting Samples Taken With the Impinger: Jour. Ind. Hyg. Toxicol., vol. 21, 1939, pp. 226-230.
28/ Ohlheiser, H. R., and Lawrence, L. B., Dust Counting - A Simplified Technic: Jour. Ind. Hyg. Toxicol., vol. 22, 1940, pp. 472-476.
29/ Moir, J., Work cited in footnote 15.
30/ Annetts, M., and Leitch, J. D., The Effect of Various Physical Factors on the Counting of Silica Dust Suspended in Water: Jour. Ind. Hyg. Toxicol., vol. 18, 1936, pp. 98-105.

Cells 0.25 Mm. in Depth

The cell 0.25 mm. in depth shown in figure 2 was developed for counts on dust about 1934 by T. Hatch,[31] then of the Harvard School of Public Health. The cell has a rectangular floor the length of the cell and 0.25 mm. below the top of the cover glass supports at the sides of the cell.

These cells have been used to a limited extent for regular and experimental counts on dust.

Cells 3 Mm. in Depth

The 3-mm.-deep cell shown in figure 2 was developed for examination of living specimens in very large hanging drops or in agar blocks. It consists of a square glass plate about 3 mm. thick, with a 16-mm.-diameter hole in the center, cemented to a 75 x 25 mm. glass slide about 2 mm. thick.

It has been used to a very limited extent for experimental dust counts.

Cells of Other Depths

Cells of other depths have been used by the Bureau of Mines, Annetts and Leitch,[32] and probably others, mainly for experimental dust counts. The Bureau of Mines has used cells 0.4 mm. deep for such purposes. Annetts and Leitch describe the use of cells 0.7 and 0.9 mm. deep. South African investigators, as previously mentioned, have described the use of cells 2 mm. deep for regular dust counts.

Some, possibly most, of these cells have been similar in shape to the Sedgwick-Rafter cells shown in figure 1 (A).

SUMMARY

This report contains information on the cells or containers used to hold a known small depth of the dust-containing liquid from impinger, filter-paper, and other dust samples while the particles revealed by a microscope are counted.

Information is given on the kinds of cells available, the kinds used, actual depth of the cells, and methods of making simple and inexpensive ones.

A new dual cell, essentially containing in one unit the two cells in which counts on impinger, filter-paper, and other samples are made, is described.

31/ Hatch, T., and Pool, C. L., Quantitation of Impinger Samples by Dark-field Microscopy: Jour. Ind. Hyg., vol. 16, 1934, pp. 177-191.
32/ Annetts, M., and Leitch, J. D., Work cited in footnote 30.